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Insulin and estrogen independently and differentially reduce macronutrient intake in healthy men

Running title: Insulin, estrogen and macronutrient intake

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Central nervous insulin inhibits food intake, but this effect has been found to be relatively reduced in female animals and women. This sex-specific pattern has been suspected to arise from a modulating influence of estrogen signaling. We therefore assessed in healthy young men whether estradiol administration interacts with the anorexigenic effect of intranasal insulin delivery to the brain. According to a 2x2 design, two groups of men (each *n*=16) received a 3-day transdermal estradiol (100 µg/24 h) or placebo pre-treatment and on two separate mornings were intranasally administered 160 IU regular human insulin or placebo before their free-choice food intake from an ad libitum breakfast buffet was assessed. Estrogen treatment induced a more than four-fold increase in serum estradiol concentrations and suppressed serum testosterone concentrations by around 70%. Independent of estradiol administration, intranasal insulin reduced the intake of carbohydrates during breakfast, attenuating in particular the consumption of sweet, palatable foods. Estradiol treatment per se decreased protein consumption. Results indicate that estrogen does not interact with central nervous insulin signaling in the control of eating behavior in humans. Insulin and estradiol rather exert independent inhibiting effects on macronutrient intake in men.

Keywords: Estrogen, insulin, ingestive behavior, macronutrients, carbohydrate, brain, intranasal administration.

Trial registration number (DRKS-ID): DRKS00007175.

The pancreatic hormone insulin, in addition to its peripheral effects, is known to impact central nervous functions including the control of energy metabolism (1,2). The direct application of insulin to the brain via intracerebroventricular (icv.) infusion in animals and, respectively, intranasal administration in humans (3) has been shown to decrease food intake and body weight in mice (4), rats (5), baboons (6), and men (7,8). These anorexigenic effects of central nervous insulin appear to display a marked preponderance in male compared to female organisms (7-9). In humans, intranasal insulin administration was found to acutely reduce food intake in men but not women (7) and during long-term treatment reduced body fat content in male but not age-matched female subjects (8). Similarly, in contrast to male rats, intact female animals do not reduce their food intake during icv. insulin treatment (9). Sensitivity to the anorexigenic effect of icv. insulin, however, can be induced in female animals by ovariectomy associated with a reduction in plasma estradiol concentrations, and vice versa, estrogen-treated male rats are no longer susceptible to the feeding-inhibiting effect of the hormone (10), suggesting that sex-related differences in estrogen signaling modulate the impact of central nervous insulin on eating behavior. Nevertheless, while not affecting food intake in the fasted state, intranasal insulin administered postprandially inhibits snack intake in women (11), indicating that the peptide can basically decrease calorie consumption also in females.

Against this background and considering that estrogen itself has been reported to attenuate energy intake and body weight in animals (12), we investigated the relevance of estrogen, insulin and their interaction in the acute regulation of eating behavior in humans. To this end, we assessed the effect of intranasal insulin on food intake in healthy young men who were pre-treated for 3 days with transdermal estradiol or placebo, hypothesizing that increasing the circulating concentrations of estrogen decreases the susceptibility of males to the anorexigenic effect of insulin.

Research Design and Methods

Subjects, Design and Procedure

Thirty-two healthy men aged between 18 and 31 years (mean age, 23.94 ± 0.52 years; mean body mass index, BMI, 22.80 ± 0.36 kg/m^2) participated in the experiment. Current illness was excluded by clinical examination and subjects were free of medication and non-smokers. They gave written informed consent to the study that conformed to the Declaration of Helsinki as revised in 2008 and was approved by the local Ethics Committee on Research Involving Humans.

Study design and experimental procedures are summarized in Figure 1. According to a 2x2 design, subjects were randomly assigned to two groups (each n = 16) that were treated with either estradiol ('estrogen patch' group; 24.38 ± 0.93 years; 22.62 ± 0.50 kg/m^2) or placebo ('placebo patch'; 23.50 ± 0.49 years, $P > 0.41$; 22.98 ± 0.54 kg/m^2, $P > 0.61$) each time before participating in two individual experimental sessions where they received intranasal insulin and placebo, respectively. Three days before each test session, subjects attended our laboratory at 1700 h. In the participants of the estrogen patch group, two transdermal estradiol patches (Estradot 50 ®, Novartis Pharma, Nuremberg, Germany) were applied to the abdomen, delivering a total dose of 100 µg estradiol per 24 hours according to the manufacturer. Participants of the placebo patch group received two patches that looked identical to the estradiol patches but did not contain the hormone. The patches were renewed by the experimenters after 24 and 48 hours, i.e., the third pair of patches was attached on the day before and removed directly after the experiment proper. Subjects as well as experimenters were blinded with regard to both the patches and the intranasal treatment. Experimental days were separated by at last 3 weeks, and the order of conditions was balanced across subjects.

The experimental procedure on each test day was similar to our previous experiments on the acute effects of intranasal insulin on food intake (7,13). All subjects remained fasted

and abstained from drinking caloric beverages after 2200 h on the evening before testing. After the subject's arrival at the laboratory at around 0800 h, a venous cannula was inserted into the subject's non-dominant arm for the collection of venous blood and the determination of blood glucose (HemoCue B-Glucose-Analyzer, HemoCue AB, Angelholm, Sweden). Sessions started with a 60-min baseline period including blood sampling at 0815 h, 0830 h, and 0845 h, and ratings of mood and hunger. At 0900 h, subjects intranasally administered sixteen 0.1-ml puffs (8 per nostril) of insulin or placebo at 30-sec intervals, amounting to a total dose of 1.6 ml insulin (160 IU; Insulin Actrapid; Novo Nordisk, Mainz, Germany), or vehicle. At 1025 h, following post-administration blood sampling at 10-20 min intervals and further assessments of mood and hunger, a standardized free-choice breakfast buffet was offered comprising a variety of food choices (Table 1) from which subjects were allowed to eat ad libitum during the subsequent 30 min. They were not aware that their food intake was measured by weighing buffet components before and after breakfast. This procedure has been repeatedly shown to enable the precise assessment of food intake in the fasted state (7,13,14). Throughout the experiments, subjects also repeatedly underwent a battery of cognitive tests unrelated to the topic of the present study (data not shown). After final blood sampling and another assessment of mood and hunger, subjects were asked in a short interview which patch (estrogen/placebo) and which spray (insulin/placebo) they thought to have received. In these interviews, none of the subjects reported adverse side effects.

Hormonal and psychometric assessments

Blood samples were centrifuged immediately, and serum was stored at -80°C. Concentrations of estradiol, testosterone, insulin, C-peptide, and cortisol (all sampling time points) as well as LH and FSH (first and third baseline time points) were determined by Immulite (DPC, Los Angeles, CA). Hunger, thirst and tiredness were rated on 9-point scales twice during baseline, at 20-30 min intervals after spray administration, and after the test breakfast. In parallel, mood

was assessed with 5-point scales covering the categories good/bad mood, alertness/sleepiness and calmness/agitation (MDBF; ref. 15). Blood pressure and heart rate were measured before and approximately 10 min after spray administration.

Statistical analyses

Analyses were performed with SPSS® Statistics Version 21 (IBM, Armonk, US) and based on repeated-measures ANOVA with the between-subjects factor "Group" (estrogen patch vs. placebo patch) and the within-subject factors "Treatment" (insulin vs. placebo), "Time", "Macronutrient" and "Taste" (i.e., neutral/sweet/savoury) as appropriate. Significant ANOVA interactions were specified by Student's *t* tests. All data are presented as means ± SEM. A *P* value less than 0.05 was considered significant.

Results

Hormonal parameters

Transdermal estrogen in comparison to placebo treatment induced a four-fold increase in plasma estradiol concentrations ($F(1,30)=84.50$, $P<0.0001$) and a 70% decrease in testosterone ($F(1,30)=77.00$, $P<0.0001$ for Group; Figure 2A/B). Both estradiol and testosterone displayed a postprandial drop after breakfast intake ($P<0.0001$ for Time). Intranasal insulin did not display any modulatory influence on these parameters (all $P>0.44$). Concentrations of LH and FSH measured during baseline were strongly suppressed after estrogen treatment (both $P<0.01$ for Group, Figure 2C).

Parameters of glucose metabolism and cortisol concentrations did not differ between conditions during baseline (all $P>0.09$) and were generally not affected by estrogen treatment (all $P>0.19$ for respective interactions). Intranasal insulin administration induced a slight decrease in blood glucose concentrations ($F(3,98)=3.81$, $P<0.02$ for Treatment × Time) that clearly remained within the euglycemic range (Figure 2D). Corresponding increases in the

concentrations of circulating insulin and C-peptide after intranasal insulin in comparison to placebo administration did not reach statistical significance ($F(2,37)=2.42$, $P<0.12$, and $F(1,33)=3.01$, $P<0.09$ for Treatment × Time; Figure 2E/F). Cortisol concentrations showed the expected circadian decline but were not affected by any of the hormonal interventions ($P>0.41$; Figure 2G).

Food intake

Across groups, insulin in comparison to placebo specifically reduced the intake of carbohydrates from the test buffet (Figure 3A; $F(1,30)=5.60$, $P<0.03$; $F(2,48)=4.39$, $P<0.03$ for Treatment × Macronutrient) against the background of comparable total food intake in both conditions ($F(1,30)=0.58$, $P>0.45$; Table 2), while the intake of fat and protein was not affected by insulin (all $P>0.48$; Figure 3A). The suppressive effect of intranasal insulin on carbohydrate intake was confirmed in covariance analyses correcting for the difference between conditions in blood glucose concentrations (expressed as the area under the curve for the period from insulin administration to breakfast; $F(1,29)=5.63$, $P<0.03$; $F(2,47)=5.70$, $P=0.01$ for Treatment × Macronutrient). The reduction in the consumption of carbohydrates was also reflected by slight decreases and increases in the consumption of food items with sweet and savoury taste, respectively ($F(2,54)=3.88$, $P<0.04$ for Treatment × Taste; Table 2). Estrogen administration in the three days preceding the experiment did not modulate the effect of intranasal insulin on food intake, neither regarding carbohydrate consumption ($F(1,30)=0.03$, $P>0.87$ for Treatment × Group) nor total intake and consumption of fat, protein, and sweet vs. savoury foods (all $P>0.52$).

Exploratory analyses restricted to subjects with a total calorie intake beyond the median of the respective placebo spray conditions, which averaged 1414 kcal, revealed that in these intense eaters ($n=8$ per estrogen and placebo patch group, respectively), intranasal insulin compared to placebo decreased not only carbohydrate consumption (670.69 ± 26.31

vs. 774.50 ± 23.52 kcal; F(1,14)=12.41, *P*<0.01), but also total food intake (1487.37 ± 67.75 vs. 1648.04 ± 50.31 kcal; F(1,14)=6.09, *P*<0.03), while fat and protein ingestion were not significantly altered (both *P*>0.08; F(2,23)=3.05, *P*>0.07 for Treatment × Macronutrient). Again, estrogen did not modulate these effects (*P*>0.45).

Independent of the intranasal treatment estrogen per se attenuated the intake of protein from the test buffet (F(1,30)=5.12, *P*=0.03 for Group; Figure 3B). Total intake and the intake of fat and carbohydrate remained unaffected by estrogen (all *P*>0.47). While protein-specificity of estrogen's anorexigenic effect was not statistically confirmed (F(2,48=0.72, *P*>0.46 for Group × Macronutrient), estrogen treatment in particular reduced the intake of savoury food items (estrogen vs. placebo patch, 297.45 ± 22.28 vs. 381.95 ± 22.28 kcal; F(1,30)=7.19, *P*<0.02) such as sliced and natural cream cheese (138.72 ± 16.11 vs. 184.93 ± 16.11 kcal, F(1,30)=4.11, *P*<0.06; 22.87 ± 7.97 vs. 44.91 ± 7.97 kcal, F(1,30)=3.83, *P*<0.06, respectively) in favour of an increase in the intake of items like hazelnut spread (100.11 ± 17.26 vs. 50.31 ± 17.26 kcal; F(1,30)=4.18, *P*=0.05). Hunger and thirst ratings were affected neither by estrogen treatment nor by insulin administration (all *P*>0.13).

Control parameters

Self-rated mood and alertness according to the MDBF adjective scale generally improved during the experiment (both *P*<0.002 for Time) but were not affected by estrogen treatment or insulin administration (all *P*>0.11). Accordingly, tiredness rated on 9-point scales decreased between morning and noon (*P*<0.001). Neither tiredness nor calmness/agitation ratings showed differences between conditions or groups (all *P*>0.10). Cardiovascular parameters were not affected by estrogen or insulin administration (all *P*>0.21). In the estrogen patch group, systolic/diastolic blood pressure measured 10 min after intranasal insulin in comparison to placebo administration was 118.33 ± 3.45 / 72.40 ± 2.36 vs. 123.88 ± 2.71 / 72.44 ± 2.59 mmHg, and 124.56 ± 2.83 / 71.06 ± 2.94 vs. 120.13 ± 3.69 / 72.25 ± 2.32 mmHg

in the placebo patch group. Heart rate in the estrogen patch group was (insulin vs. placebo) 59.67 ± 1.80 vs. 59.06 ± 2.14 bpm and 58.63 ± 2.24 vs. 59.88 ± 2.71 bpm in the placebo patch group. In the post-experimental interviews, subjects were not able to correctly indicate whether they had received estrogen or placebo patches (P>0.53) and insulin or placebo sprays (P>0.71; χ^2 tests).

Discussion

We investigated the influence of estrogen on the impact of central nervous insulin administration via the intranasal pathway in humans, which is known to exert stronger acute (7) and long-term (8) anorexigenic effects on energy homeostasis in male than female subjects. We found that quadrupling circulating estrogen concentrations in healthy young men by means of transdermal estradiol patches does not alter the suppressive effect on carbohydrate intake of intranasal insulin. This outcome stands in some contrast to animal experiments indicating that estrogen action interferes with the anorexigenic effect of brain insulin administration (9,10). Estrogen administration per se was revealed to induce a small, but discernible reduction in protein consumption, indicating that both insulin and estrogen, but in an independent fashion, have a restraining effect on the intake of macronutrients in men.

The pre-treatment of our subjects with estradiol patches worn for 3 consecutive days proved to be highly effective as evidenced by the 4-fold increase in circulating estrogen while the concentrations of LH and FSH were roughly halved and serum testosterone dropped by about 70%. The estrogen-induced reduction in the intake of proteins from the test breakfast buffet fits with animal experiments indicating that centrally administered estrogen, similar to the adiposity signal leptin (16), inhibits food intake (17,18), most likely by binding to the estrogen receptor α located among other relevant sites in the hypothalamic arcuate and ventromedial nuclei and the nucleus of the solitary tract in the hindbrain (19,20). Accordingly,

daily food intake in naturally cycling women reaches its nadir during the peri-ovulatory phase when estradiol concentrations are maximal (21-23), although the intake of specific macronutrients appears to be unaffected (24,25). Considering that testosterone has orexigenic properties (26-28), the strong estrogen-induced reduction in circulating testosterone concentrations in our study might have been another mediator of decreased calorie consumption. Although the moderate estrogen effect was evident for protein rather than fat or carbohydrate intake, it points at the potential of estrogen delivery to restrain food intake, in particular when pharmacologically enhanced to target key brain structures (29).

Irrespective of estrogen pre-treatment, intranasal insulin reduced carbohydrate intake from the test breakfast, a finding that corroborates and specifies previous observations (7) and is in line with the notion that insulin transported to the CNS acts as a negative feedback signal in the control of food intake (2,6). In accordance with related experiments (7,13,30), intranasal insulin also induced a slight, euglycemic decrease in blood glucose concentrations that presumably stemmed from a small ratio of exogenous insulin entering the circulation via the nasal mucosa. Importantly, insulin's effect on food intake was confirmed in analyses corrected for pre-breakfast changes in blood glucose concentrations, so that a peripheral mediation of the decrease in carbohydrate consumption is unlikely. In our previous study in healthy young men, intranasal insulin globally reduced food intake from a comparable free-choice breakfast buffet by around 200 kcal (7). This effect was well replicated in the intense eaters among the present subjects, although the post-hoc nature of the respective analyses leaves open the question whether intranasal insulin is particularly effective in reducing high calorie consumption. Our observation in the whole sample that insulin restrains carbohydrate intake and the ingestion of sweet food items ties in with our result in non-fasted women of an insulin-induced reduction in the intake of chocolate cookies (11). This pattern suggests that besides decreasing hunger-driven, homeostatic energy intake, insulin moreover acts in the brain to inhibit the reward-related consumption of particularly palatable foods (31), without

affecting general mood as indicated by respective rating scales. Accordingly, animal studies have shown that brain administration of insulin decreases the rewarding quality of food (32,33), presumably by suppressing mesolimbic dopaminergic signaling (34).

Contrary to our expectation, estrogen pre-treatment did not modulate the reduction in carbohydrate intake induced by intranasal insulin. In rats, the peripheral administration of estradiol at a dose of 2 μg administered every fourth day for 1 month completely blunted the reduction in 24-h food intake and body weight observed in control animals after insulin injection into the third cerebral ventricle (10). In these animals, estradiol treatment increased plasma estradiol concentrations by 60%, averaging peri-ovulatory peak concentrations of female animals (35), whereas quadrupling serum estradiol concentrations in our male subjects yielded concentrations that approximately mirrored mean values during the menstrual cycle in young women. In principle, extended administration periods and higher dosages of estradiol might be hypothesized to modulate the anorexigenic effect of insulin, but estrogen's impact on eating behavior in the present paradigm is clearly illustrated by its suppressive effect on protein intake. Thus, it seems safe to conclude that inducing peripheral estrogen concentrations in healthy young men that approximate the situation regularly found in women and, notably, induce a 70% reduction in testosterone concentrations, does not alter the brain's sensitivity to the anorexigenic impact of insulin. Different expression patterns of estrogen receptors in the male and female brain (36,37) might contribute to altered estrogen-insulin interactions in men compared to women. However, in previous experiments restricted to women (13), the difference in estrogen levels between young women receiving ethinyl estradiol-dominant contraceptives and postmenopausal women, in accordance with the present data, was not associated with differences in the response to intranasal insulin.

In sum, we demonstrate that in healthy men, central nervous insulin delivery via the intranasal route decreases carbohydrate consumption in the presence of normal and strongly elevated circulating estrogen concentrations alike. This pattern indicates that estrogen, which

itself displays moderate suppressive effects on protein intake, and insulin do not interact in the regulation of eating behavior in humans. Further investigations are needed to gain insight into neurobiological mechanisms underlying the stronger anorexigenic effect of central insulin in males than females reported in animals (9) and humans (7,8). Such studies might also contribute to the development of sex-specific, individually tailored approaches in the treatment of eating disorders.

Author contributions

R.K., V.O., J.B. and M.H. designed research; R.K., V.O., L.M., and M.H. conducted research; R.K., L.M., and M.H. analyzed data; R.K. and M.H. wrote the paper; all authors read and approved the final manuscript. M.H. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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The authors have no potential conflicts of interest relevant to this article.

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Table 1. Composition of the breakfast test buffet.

Food	Weight (g)	Energy (kcal)	Carbohydrate (g)	Fat (g)	Protein (g)
Neutral					
Whole wheat bread	165	329	63.9	2	12.1
Wheat rolls	300	857	167.6	5.4	30
White bread	30	73	14.7	0.4	2.5
Butter	75	580	0.5	62.4	0.5
Whole milk	750	495	35.3	26.8	25.4
Condensed milk	40	54	3.9	3	2.6
Sweet					
Jam	50	140	34.2	0	0.1
Hazelnut spread	40	218	22.7	12.4	2.7
Honey	40	123	30	0	0.2
Sugar	24	98	24	0	0
Fruit curd	150	148	24.8	1.2	8.8
Banana	190	167	38.1	0.3	2.2
Apple	120	71	17	0.1	0.4
Pear	190	105	23.5	0.6	0.9
Orange juice	400	173	36	1	4
Savoury					
Poultry sausage	40	74	0.1	4.3	8.3
Cervelat sausage	34	138	0.1	11.8	6.9
Sliced cheese	100	198	0	23	20.8
Cream cheese (natural)	33	87	1.1	8.2	1.8
Cream cheese (herbs)	40	84	1.2	7.2	3.2
Total	**2811**	**4312**	**538**	**170**	**133**

Breakfast was served with coffee or tea as requested by the participant.

Table 2. Food intake from the test buffet.

Food intake (kcal)	Placebo	Insulin	*P* value
Total	1401.29 ± 55.84	1365.82 ± 45.61	0.45
Neutral food	743.34 ± 40.59	696.78 ± 38.33	0.18
Wheat rolls	361.24 ± 27.81	301.79 ± 29.24	0.06
Sweet food	343.50 ± 25.58	304.08 ± 23.66	0.09
Jam	37.38 ± 6.82	25.76 ± 7.44	0.03
Hazelnut spread	89.20 ± 14.01	61.23 ± 13.01	0.03
Honey	17.32 ± 5.17	8.85 ± 3.61	0.09
Sugar	10.69 ± 2.46	6.74 ± 2.38	0.07
Savoury food	314.45 ± 21.02	364.95 ± 18.44	0.04
Cervelat sausage	67.95 ± 8.37	83.03 ± 8.17	0.10
Sliced cheese	147.46 ± 13.94	176.19 ± 11.96	0.03

Total food intake, food intake according to taste, and consumption of specific food items (all in kcal). All neutral, savoury and sweet foods contained in the test buffet are listed in Table 1. Values are means ± SEM calculated across experimental groups (placebo and estrogen patch) for the experimental conditions (placebo and insulin spray). Right column indicates *P* values for the ANOVA factor Treatment. n=32.

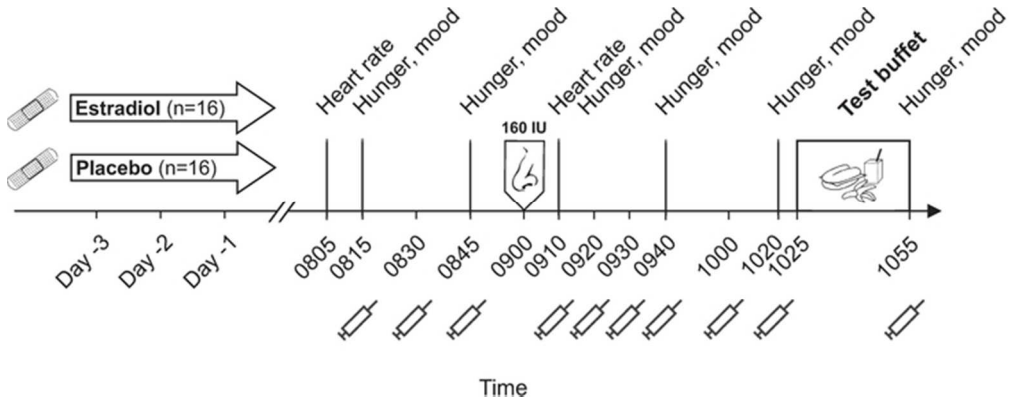
Figure legends

Figure 1. Experimental procedure. Two groups of 16 healthy men who had been pre-treated with transdermal estradiol (100 µg/24 h for three days) or placebo participated in two experimental sessions. Following a baseline period of around 60 min, they were intranasally administered 160 IU insulin and, in the other condition, placebo at 0900 h before a free-choice test breakfast buffet was offered around 85 min later. Self-rated hunger, thirst, tiredness and mood were repeatedly assessed and blood samples for the determination of glucose and hormone concentrations were obtained (syringe symbols). Heart rate and blood pressure were assessed twice.

Figure 2. Endocrine parameters. Serum concentrations of (A) estradiol, (B) testosterone, and (C) LH and FSH, (D) blood glucose concentrations, and serum concentrations of (E) insulin, (F) C-peptide, and (G) cortisol. Experiments were performed in two groups of 16 men each who had received 3 days of transdermal estradiol (100 µg/24 h; squares) or placebo pre-treatment (circles) before participating in experimental sessions starting with baseline measurements followed by the intranasal spray administration of 160 IU insulin (filled symbols, solid lines) and placebo (empty symbols, dashed lines), respectively, at 0900 h (arrow mark). LSH and FSH represent the average of the 0815 h and 0845 baseline measurements. Values are means ± SEM. *** $P < 0.001$, ** $P < 0.01$ for the ANOVA factors Group (panel C) and Treatment (D), respectively.

Figure 3. Food intake from the test buffet. Intake of macronutrients (kcal) from a standardized free-choice breakfast buffet presented 85 min after intranasal spray administration of 160 IU insulin and placebo, respectively, in healthy men who had received 3 days of transdermal estradiol (100 µg/24 h) or placebo pre-treatment (each n=16) before the experimental day. (**A**) Macronutrient intake in the insulin (black bars) and the placebo spray

conditions (white bars) collapsed across the estrogen and placebo patch groups. (**B**) Macronutrient intake in the estradiol (gray bars) and the placebo patch (white bars) groups collapsed across the insulin and placebo spray conditions. Values are means ± SEM. * $P <$ 0.05 for the ANOVA factors Treatment (panel A) and Group (B), respectively.



Experimental procedure. Two groups of 16 healthy men who had been pre-treated with transdermal estradiol (100 µg/24 h for three days) or placebo participated in two experimental sessions. Following a baseline period of around 60 min, they were intranasally administered 160 IU insulin and, in the other condition, placebo at 0900 h before a free-choice test breakfast buffet was offered around 85 min later. Self-rated hunger, thirst, tiredness and mood were repeatedly assessed and blood samples for the determination of glucose and hormone concentrations were obtained (syringe symbols). Heart rate and blood pressure were assessed twice.
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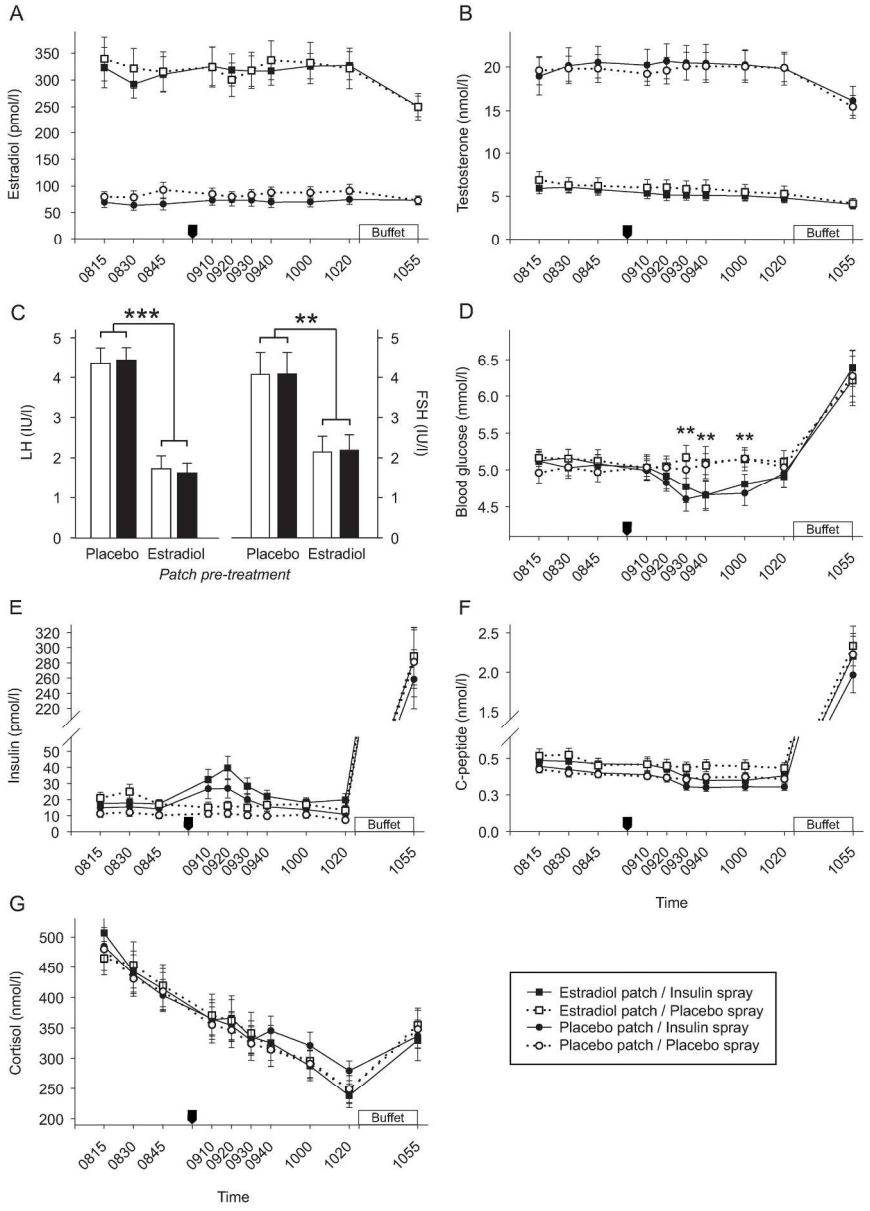

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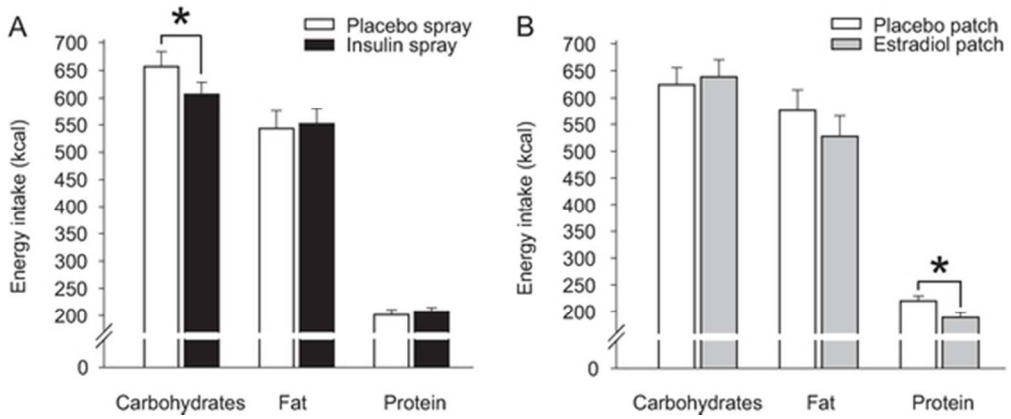

Food intake from the test buffet. Intake of macronutrients (kcal) from a standardized free-choice breakfast buffet presented 85 min after intranasal spray administration of 160 IU insulin and placebo, respectively, in healthy men who had received 3 days of transdermal estradiol (100 µg/24 h) or placebo pre-treatment (each n=16) before the experimental day. (A) Macronutrient intake in the insulin (black bars) and the placebo spray conditions (white bars) collapsed across the estrogen and placebo patch groups. (B) Macronutrient intake in the estradiol (gray bars) and the placebo patch (white bars) groups collapsed across the insulin and placebo spray conditions. Values are means ± SEM. * $P < 0.05$ for the ANOVA factors Treatment (panel A) and Group (B), respectively.
51x20mm (300 x 300 DPI)